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In silico assessment of human health risks caused by cyanotoxins from cyanobacteria

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Abstract: Harmful algal blooms (HABs) that are formed by cyanobacteria have become a serious issue worldwide in recent years. Cyanobacteria can release a type of secondary metabolites called cyanotoxins into aquatic systems which may indirectly or directly provide health risks to the environment and humans. Cyanotoxins provide some of the most powerful natural poisons including potent neurotoxins, hepatotoxins, cytotoxins, and endotoxins that may result in environmental health risks, and long-term morbidity and mortality to animals and humans. In this research, we used the chemcomputational tool Molinspiration for molecular property predictions, Pred-hERG 4.2 web software for cardiac toxicity prediction, and Pred-Skin 2.0 web software for predicting skin sensitization. We are predicting some toxicological aspects of cyanobacteria here using chemcomputational tools with the hypothesis that cyanotoxins are providing a risk to human health. We are using the tool Pred-hERG 4.2 to predict hERG channel blocking potential and the Pred-skin tool to predict skin sensitization due to cyanotoxins. The potential of anatoxin, ambigol, the microcystin group, and lyngbyatoxin A, lyngbyatoxin B, nodularin-R, and saxitoxin were predicted to cause skin sensitization in the final results (consensus model). Anatoxin-a and lyngbyatoxin were predicted to allow GI absorption and blood–brain barrier penetration. Among the 20 predicted cyanotoxins only aeruginosin 103-A, ambigol A, and ambigol were predicted by Pred-hERG 4.2 according to the applicability domain results as potential cardiotoxins with weak or moderate potency. Lyngbyatoxin shows activity through the GPCR ligand and protease, kinase, and enzyme inhibitor.

Introduction

Cyanobacteria occur in aquatic environments such as freshwaters (rivers, lakes, ponds, etc.), brackish waters (Hamilton *et al.*, 2014), and the oceans (Schaefer *et al.*, 2020). They are primary producers and play important roles in aquatic ecosystems, converting nitrogen into organic forms that can be used as macronutrients by eukaryotes, and oxygenic photosynthesis (Gademann and Portmann, 2008; Harke *et al.*, 2016; Wood *et al.*, 2007). However, climate change and eutrophication can result in harmful algal blooms (HABs) that are caused by excessive growth of cyanobacteria, and the release of high concentrations of toxic secondary metabolites called cyanotoxins becomes a serious threat. This holds for other organisms as well as for the safety of drinking water, aquatic food sources (Agasild *et al.*, 2019), and public health (Aráoz *et al.*, 2010).

Cyanotoxins provide toxic compounds that may affect the environment, animals, and humans by exposure through, e.g., seafood and aquaculture consumption, water contact during recreation, and drinking water (Meriluoto *et al.*, 2017; Bukaveckas *et al.*, 2018). Cyanotoxins can cause serious public health issues after long-term exposure through contaminated drinking water and recreational contact in fresh- and saltwater systems. Seafood consumption is another concern (Stone and Bress, 2007). Exposure to cyanobacteria can cause health effects in

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humans. These may include vomiting, flu-like symptoms, rashes, nausea, fever, gastroenteritis, blistered mouth, skin, eye, and ear irritation, visual disturbances, abdominal pain, and systemic effects such as hepatic failure, neurological damage, and death (Brown et al., 2018; Codd *et al*., 2005; Kubickova et al., 2019). Skin irritation can result from contact with toxic cyanobacteria (Pilotto *et al*., 2004). Cyanobacteria can also cause allergic reactions with symptoms like hives, conjunctivitis, and asthma (Farrer *et al*., 2015).

Cyanobacterial toxigenic compounds provide major waterborne health risks globally (Schaefer *et al*., 2020). Several toxins appear to be confined to specific cyanobacteria, but some cyanobacteria are known to produce a variety of toxic compounds (Haque *et al*., 2017). Cyanotoxins contain a variety of chemical compounds that differ by their chemical structure. Toxicological endpoints can be separated by their effects on liver toxins, neurotoxins, dermatotoxins, and endotoxins (Brown et al., 2018; Mankiewicz *et al*., 2003; Haque *et al*., 2017) (Tab. 1). Different approaches try to classify cyanotoxins, such as their respective biological sources, toxicity, molecular mass, and structural characteristics (Johnson *et al*., 2011). Reproductive and developmental toxicity, as well as carcinogenicity, are as yet not conclusively connected to cyanotoxins. This holds for marine systems but also for freshwater ones, where cyanotoxins represent an emerging health concern (IJCH Report, 2017).

Cyanotoxins can cause alimentary poisoning in humans and animals with different syndromes such as: Diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), and azaspiracid shellfish poisoning (ASP) (Bigalke and Rummel, 2005). Several toxic compounds were found in cyanobacteria worldwide from oceanic environments and reservoirs and freshwater lakes worldwide (Huang and Zimba, 2019). Most threatening are neurotoxins affecting the nervous system (e.g., tetrodotoxin and saxitoxin), providing considerable potential as toxins even for military use (Pitschmann and Hon, 2016). For example, saxitoxin (STX) binds to the voltage-gated Na^+ channel, subsequently blocking neuronal transmissions (Arnich and Thebault, 2018). It is the most studied and the first known compound to cause paralytic shellfish poisoning (PSP). The aerosol of the unstable saxitoxin degrades per minute at a rate of 17% (Jansson and Åstot, 2015). Saxitoxin is able to overcome the dermal barrier (similar to brevetoxin, anatoxin, and tetrodotoxin). However, it does not reach the efficacy of organophosphate as a chemical warfare agent (CWA) with another example of cyanobacterial neurotoxin, β-N-methylamino-l-alanine (BMAA), which is a cyanobacterial neurotoxin (Kubo *et al*., 2008). The toxins of several cyanobacterial genera also showed antineoblastic potential in human cell lines and promising results with respect to human adenocarcinomas (Zanchett and Oliveira-Filho, 2013).

We are applying chemoinformatics here as a search for chemical information that transfers chemical data to simulations. *In silico* approaches in toxicological studies make use of informational techniques that allow the predictions of toxic effects of cyanotoxins. We are using chemcomputational tools here following the hypothesis that cyanotoxins are providing risks to human health.

Materials and Methods

Data preparation and analysis

We accessed 21 cyanotoxins through the database of PubChem (https://pubchem.ncbi.nlm.nih.gov/) and a literature report. Isomeric SMILES notations of cyanotoxins collected from PubChem are presented in Tab. 2, and structures sketched by software ChemDraw 18.1 (https://www.perkinelmer.com/au/category/chemdraw) are presented in Fig. 1. Linear kernels like SMILES were used for our toxicity predictions since their similarity functions are computationally more efficient (Frenzel *et al*., 2017).

ADME prediction

We were using Swiss ADME online software (http://www.swissadme.ch) in our toxicity discovery to predict parameters related to "absorption, distribution, metabolism, and excretion" (ADME) such as medicinal chemistry, druglike nature, friendliness of one or multiple small molecules as well as to compute parameters related to their physicochemistry. Swiss ADME enables assessment of ADME parameters of drug candidates and small molecules and provides information that allows early risk assessment in the drug development process. Notably, Swiss ADME provides a platform to assess the drug-likeness of oral bioavailability through Lipinski's rule of five (Jayaseelan *et al*., 2012; Lipinski, 2000). Swiss ADME is a more recent and comprehensive site run by the Swiss Institute of Bioinformatics (SIB), which provides bioinformatics services and resources for scientists worldwide. SIB has over 800 scientists and 65 bioinformatic teams from major Swiss research and higher education institutes (Daina *et al*., 2017).

Cardiac toxicity

One form of the Long QT syndrome (LQTS) with a lack of repolarization of the heart after a heartbeat was disturbed, is related to a defect of the hERG protein that affects the K^+ channel functioning during cardiac electric excitability. Identification of a hydrophobic area, which represents the putative interface and tight binding region with the interface of the K^+ channel, became possible by the screening for mutagenesis of the domain surface. Once this hydrophobic domain of the channel is present, the rate of deactivation is slowed down (Cabral *et al*., 1998). The first three-dimensional model of a eukaryotic domain called Per-Arnt-Sim (PAS) is structurally similar to a yellow protein functioning as a bacterial light sensor.

Skin sensation

In order to confirm or reject the sensitization effect of cyanobacterial compounds evaluated here, we used Pred-Skin 3.0 web online software (http://labmol.com.br/predskin/), available as an online software program (Braga *et al*., 2017).

Bioactivity prediction

The bioactivity potential of cyanotoxins was predicted by calculating the activity score for the ion channel modulator, the GPCR ligand, the nuclear receptor ligand, and the inhibitors of enzymes, proteases, and kinases (Jabeen and Ranganathan, 2019). All the above parameters were tested

TABLE 1

Health risks provided by toxins from cyanobacteria

Cyanotoxin class	Toxigenic genera	Structure	Activity	Syndrome	References
Hepatotoxins					
Cylindrospermopsins (3)	Anabaena, Aphanizomenon, Cylindrospermopsis Raphidiopsis, Umezakia	Guanidine alkaloids	Cytochrome P450 and Glutathione and protein synthesis	Gastroenteritis, intestine damage, liver, kidney	Bláha *et al.* (2009)
Microcystins (>100)	Anabaena, Anabaenopsis, Hapalosiphon, Microcystis, Planktothrix	Cyclic heptapeptides	Protein phosphatase type 1 and 2A inhibition	Liver damage	Bláha *et al.* (2009); Rinehart *et al.* (1988)
Nodularins (10)	Nodularia	Cyclic pentapeptides	Protein phosphatase type 1 and 2A inhibition	Liver necrosis	Rinehart *et al.* (1988)
Neurotoxins					
Anatoxins (3)	Anabaena, Aphanizomenon, Cylindropermum, Oscillatoria, Placktothrix, Raphidiopsis	Alkaloid	Irreversible inhibition of acetylcholinesterase	Hypoxia, respiratory arrest	Bláha *et al.* (2009); Méjean *et al.* (2014); Aráoz *et al.* (2010)
Saxitoxins (>60)	Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Planktothrix	Carbamate alkaloids	Sodium channel blocker in axons	Abdominal pain, eye irritation, fever, rashes	Lago *et al.* (2015); Hackett *et al.* (2013); Tsuchiya *et al.* (2015)
Dermatotoxins					
Lyngbyatoxins (>8)	Lyngbya, Oscillatoria, Schizotrix,	Alkaloid	Potentiation of protein kinase C (PKC) acts as tumor blocker; Seaweed dermatitis, Carcinogen, Blister agent	Dermatitis	Jiang *et al.* (2014)
Aplysiatoxins	Lyngbya, Oscillatoria, Schizotrix	Alkaloids	Potentiation of protein kinase C (PKC)	Irritant	Han *et al.* (2018)
Endotoxins					
Lipopolysaccharides	All cyanobacteria	Lipopolysaccharides	Inflammatory agents, gastrointestinal irritants	Chills, fever, myalgia, vomiting	Bláha *et al.* (2009)
Not identified					
Ambigol (3)	Fischerella	Chlorinated aromatic compounds	Embryo development (zebrafish *Danio rerio*)	–	Wright *et al.* (2006); Manning and Nobles, 2017
Aeruginosins (>15)	Microcystis, Nostoc, Oscillatoria, Planktothrix	–	Protein inhibitor	–	Manning and Nobles, 2017

TABLE 2

Isomeric SMILES of cyanobacterial toxins

Cyanotoxin class	Cyanotoxin name	PubChem CID	Formula	Isomeric SMILES
Aeruginosins	Aeruginosin 103-A	10009777	$C_{35}H_{48}N_6O_8$	CCO[C@@H]1[C@H](CCCN1C(=N)N)NC(=O)C2CC3CCC(CC3N2C(=O)[C@@H](CC4=CC=C(C=C4)O)NC(=O)[C@@H](CC5=CC=C(C=C5)O)O)O
	Aeruginosin 98-b	444346	$C_{29}H_{46}N_6O_9S$	CC[C@@H](C)[C@H](C(=O)N1[C@H]2C[C@@H](CC[C@H]2C[C@H]1C(=O)NCCCCN=C(N)N)OS(=O)(=O)O)NC(=O)[C@@H](CC3=CC=C(C=C3)O)O
Anatoxins	Anatoxin-a	3034748	$C_{10}H_{15}NO$	CC(=O)C1=CCC[C@@H]2CC[C@H]1N2
	Anatoxin-a(s)	114989	$C_7H_{17}N_4O_4P$	CN(C)C[C@H]1CN=C(N1OP(=O)(O)OC)N
Ambigol	Ambigol A	475341	$C_{18}H_8Cl_6O_3$	C1=CC(=C(C=C1Cl)Cl)OC2=C(C=C(C(=C2O)C3=C(C(=CC(=C3)Cl)Cl)O)Cl)Cl
	Ambigol B	475342	$C_{18}H_8Cl_6O_3$	C1=CC(=C(C=C1Cl)Cl)OC2=C(C(=C(C=C2Cl)Cl)OC3=C(C=C(C=C3)Cl)Cl)O
	Ambigol C	5276614	$C_{18}H_8Cl_6O_3$	C1=CC(=C(C=C1Cl)Cl)OC2=CC(=C(C(=C2Cl)O)Cl)OC3=C(C=C(C=C3)Cl)Cl
Aplysiatoxins	Aplysiatoxin	46173823	$C_{32}H_{47}BrO_{10}$	C[C@@H]1CC([C@@]23CC([C@@H]([C@H](O2)[C@@H](C)CC[C@@H](C4=C(C=CC(=C4)O)Br)OC)C)OC(=O)C[C@@H](OC(=O)C[C@@]1(O3)O)[C@@H](C)O)(C)C
Cylindrospermopsins	Deoxycylindrospermopsin	11280999	$C_{15}H_{21}N_5O_6S$	C[C@H]1[C@H](C[C@@H]2C[C@@H](NC3=NC[C@H]1N23)CC4=CC(=O)NC(=O)N4)OS(=O)(=O)O
	7-Epi-cylindrospermopsin	42628600	$C_{15}H_{21}N_5O_7S$	C[C@H]1[C@H](C[C@@H]2C[C@@H](NC3=NC[C@H]1N23)[C@H](C4=CC(=O)NC(=O)N4)O)OS(=O)(=O)O
Lipopolysaccharides	Lipopolysaccharide	11970143	$C_{205}H_{366}N_3O_{117}P_5$	CCCCCCCCCCCCCCCC1[C@H](OC([C@H]([C@@H]1OC(=O)CC(CCCCCCCCCCC)O)O)CO[C@H]2[C@H](C([C@@H](C(O2)CO[C@@]3(CC([C@H](C(O3)C(CO)O)O[C@H]4[C@@H](C([C@@H](C(O4)C(CO)O)OP(=O)(O)OP(=O)(O)OCCN)O[C@@H]5[C@@H](C([C@@H](C(O5)C(CO[C@@H]6[C@@H](C([C@](CO6)(C(CO)O)O)O)O)O)OP(=O)(O)O)O[C@@H]7C(C([C@@H](C(O7)CO[C@@H]8C(C([C@H](C(O8)CO)O)O)O)O)O[C@@H]9C(C([C@H](C(O9)CO)O)O)O[C@@H]1C(C([C@@H](C(O1)CO)O[C@@H]1C(C([C@H](C(O1)CO)O)OC1[C@@H](C(C([C@@H](O1)C)O[C@@H]1C(C([C@@H](C(O1)CO)O)O[C@@H]1C(C[C@H](C(O1)C)O)O)O[C@@H]1C(C([C@H](C(O1)CO)O)O)O)O)O)O)O)O)O[C@@H]1[C@H](C([C@@H](C(O1)CO)O)O)NC(=O)C)O)O)O[C@@]1(CC([C@H](C(O1)C(CO)O)O)O[C@@]1(CC([C@H](C(O1)C(CO)O)O)O)C(=O)O)C(=O)O)C(=O)O)OP(=O)(O)O)OC(=O)CC(CCCCCCCCCCC)OC(=O)CCCCCCCCCCCCCC)NC(=O)CC(CCCCCCCCCCC)OC(=O)CCCCCCCCCCC)CP(=O)(O)O

Table 2 (continued).

Cyanotoxin class	Cyanotoxin name	PubChem CID	Formula	Isomeric SMILES
Lyngbyatoxins	Lyngbyatoxin A	91706	$C_{27}H_{39}N_3O_2$	CC(C)[C@H]1C(=O)N[C@@H](CC2=CNC3=C(C=CC(=C23)N1C)[C@](C)(CCC=C(C)C)C=C)CO
	Lyngbyatoxin B	131589	$C_{27}H_{39}N_3O_3$	CC(C)C1C(=O)NC(CC2=CNC3=C(C=CC(=C23)N1C)C(C)(CCC(C(=C)C)O)C=C)CO
	Lyngbyatoxin C	6441239	$C_{27}H_{39}N_3O_3$	CC(C)[C@H]1C(=O)N[C@@H](CC2=CNC3=C(C=CC(=C23)N1C)[C@](C)(C/C=C/C(C)(C)O)C=C)CO
Microcystins	Microcystin-LR	445434	$C_{49}H_{74}N_{10}O_{12}$	C[C@H]1[C@@H](NC(=O)[C@@H](NC(=O)[C@H]([C@@H](NC(=O)[C@@H](NC(=O)[C@H](NC(=O)C(=C)N(C(=O)CC[C@@H](NC1=O)C(=O)O)C)CC(C)C)C(=O)O)C)CCCN=C(N)N)/C=C/C(=C/[C@H](C)[C@H](CC2=CC=CC=C2)OC)/C
	Microcystin-RR	6438357	$C_{49}H_{75}N_{13}O_{12}$	C[C@H]1[C@@H](NC(=O)[C@@H](NC(=O)[C@H]([C@@H](NC(=O)[C@@H](NC(=O)[C@H](NC(=O)C(=C)N(C(=O)CC[C@@H](NC1=O)C(=O)O)C)CCCN=C(N)N)C(=O)O)C)CCCN=C(N)N)/C=C/C(=C/[C@H](C)[C@H](CC2=CC=CC=C2)OC)/C
	Microcystin-YR	6437088	$C_{52}H_{72}N_{10}O_{13}$	C[C@H]1[C@@H](NC(=O)[C@@H](NC(=O)[C@H]([C@@H](NC(=O)[C@@H](NC(=O)[C@H](NC(=O)C(=C)N(C(=O)CC[C@@H](NC1=O)C(=O)O)C)CC2=CC=C(C=C2)O)C(=O)O)C)CCCN=C(N)N)/C=C/C(=C/[C@H](C)[C@H](CC3=CC=CC=C3)OC)/C
	Microcystin-LA	6437382	$C_{46}H_{67}N_7O_{12}$	C[C@H]1[C@@H](NC(=O)[C@@H](NC(=O)[C@H]([C@@H](NC(=O)[C@@H](NC(=O)[C@H](NC(=O)C(=C)N(C(=O)CC[C@@H](NC1=O)C(=O)O)C)CC(C)C)C(=O)O)C)C)/C=C/C(=C/[C@H](C)[C@H](CC2=CC=CC=C2)OC)/C
Nodularins	Nodularin	14217092	$C_{41}H_{60}N_8O_{10}$	C/C=C\1/C(=O)N[C@H]([C@@H](C(=O)N[C@H](C(=O)N[C@H]([C@@H](C(=O)N[C@H](CCC(=O)N1C)C(=O)O)C)/C=C/C(=C/[C@H](C)[C@H](CC2=CC=CC=C2)OC)/C)CCCN=C(N)N)C)C(=O)O
	Nodularin-R	45483039	$C_{40}H_{58}N_8O_{10}$	C/C=C/1\C(=O)N[C@H]([C@@H](C(=O)N[C@H](C(=O)N[C@H]([C@@H](C(=O)N[C@H](CCC(=O)N1)C(=O)O)C)/C=C/C(=C/[C@H](C)[C@H](CC2=CC=CC=C2)OC)/C)CCCN=C(N)N)C)C(=O)O
Saxitoxins	Saxitoxin	56947150	$C_{10}H_{17}N_7O_4$	C1CN2C(=N[C@H]([C@H]3[C@]2(C1(O)O)NC(=N3)N)COC(=O)N)N

by applying the drug-likeness score of Molinspiration software (www.molinspiration.com).

Results

ADME prediction

Anatoxin-a and Lyngbyatoxin were predicted to have GI absorption and blood–brain barrier (BBB) penetration (Tab. 3).

Cardiac toxicity

Among the 20 predicted cyanotoxins only aeruginosin 103-A, ambigol A, and ambigol B have cardiotoxic potential and weak or moderate potency as predicted by Pred-hERG 4.2 according to the applicability domain results (Tab. 4).

Skin sensitization

Anatoxin, ambigol, the microcystin group, lyngbyatoxin A, lyngbyatoxin B, nodularin-R, and saxitoxin were predicted to have the potential to cause skin sensitization in the final results (consensus model) (Tab. 5).

Bioactivity

The calculated values of the isolated compound endpoints and the drug-likeness scores are summarized in Tab. 6. Lyngbyatoxin, as

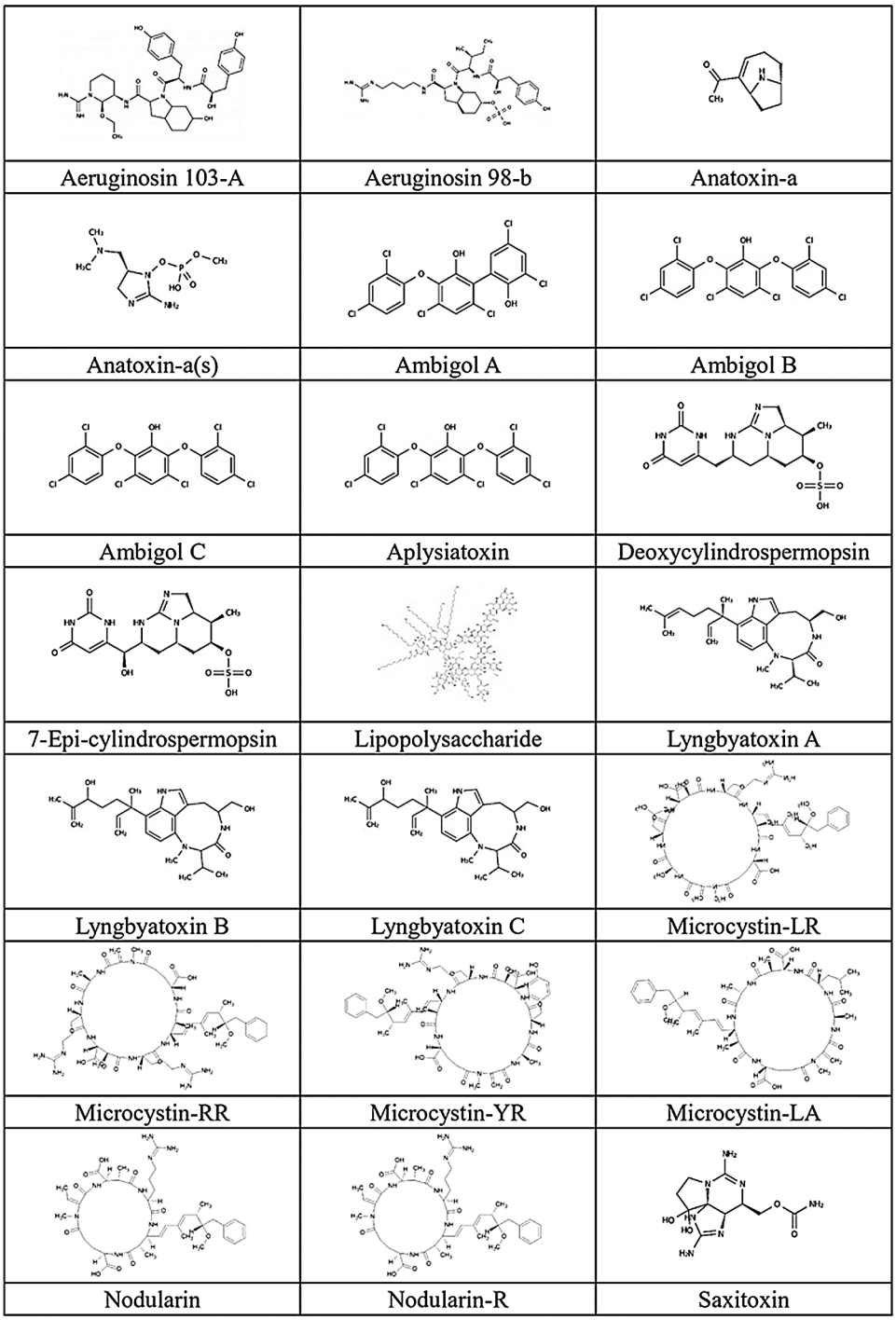


FIGURE 1. Chemical structure of cyanotoxins.

an enzyme inhibitor, shows inhibition activity against proteases and kinases through the GPCR ligand and the inhibitors of enzyme, kinase, and protease.

Discussion

Anatoxin-a and Lyngbyatoxin were predicted to have GI absorption and BBB penetration (Tab. 3). The BBB maintains homeostasis of the Central Nervous System (CNS), which is provided by the permeability BBB (Gao *et al.*, 2017).

The inability of most compounds to cross the BBB is a major limitation to effectively treat diseases in the central nervous system (Alexander *et al.*, 2019). The BBB is a highly evolved microvascular system comprised of brain endothelial cells (ECs) lining the vascular lumen, pericytes in the basal lamina, and associating astrocytic end-feet, microglia, and neurons. This cellular architecture forms functional neurovascular units that regulate molecular trafficking between the blood and the brain (Zhang *et al.*, 2019).

ADME prediction

The extent of binding can greatly influence The ADME (adsorption, distribution, metabolism, and excretion) profile that can greatly be affected by toxin binding. The evaluation

TABLE 3

ADME prediction results of 20 cyanotoxins

Molecule	GI absorption	BBB penetration	Skin permeation log Kp (cm/s)
Aeruginosin 103-A	Low	No	−9.25
Aeruginosin 98-b	Low	No	−9.85
Anatoxin-a	**High**	**Yes**	−6.71
Anatoxin-a(s)	**High**	No	−10.96
Ambigol A	Low	No	−3.49
Ambigol B	Low	No	−3.31
Ambigol C	Low	No	−3.83
Aplysiatoxin	Low	No	−6.96
Deoxycylindrospermopsin	Low	No	−9.76
7-Epi-cylindrospermopsin	Low	No	−10.64
Lyngbyatoxin A	**High**	**Yes**	−4.56
Lyngbyatoxin B	**High**	No	−5.49
Lyngbyatoxin C	**High**	No	−5.95
Microcystin-LR	Low	No	−10.74
Microcystin-RR	Low	No	−12.79
Microcystin-YR	Low	No	−11.1
Microcystin-LA	Low	No	−9.37
Nodularin	Low	No	−10.11
Nodularin-R	Low	No	−10.15
Saxitoxin	Low	No	−11.41

of biological substance effects very much depends on gastrointestinal absorption (GI absorption). In the evaluation of the biological effects of substances, this is a key criterion (Diukendjieva *et al.*, 2019). Cost-effective and reliable and cost-effective bioavailability studies early in the drug discovery process can lead to an improvement in the success rate for compounds entering clinical trials (Fabini and Danielson, 2017).

Cardiotoxicity prediction

Pred-hERG 4.2 server (http://labmol.com.br/predherg) predicts the probability maps where compounds block the K^+ ion channel coded by a human Ether-à-go-go-Related Gene (hERG) (Tab. 4). The results show that not all tested marine cyanobacterial toxins are hERG blockers in a multiclass prediction. All tested cyanobacterial compounds are hERG blockers as predicted by the Pred-hERG binary prediction, except anatoxin-A, BMAA, and hypoxanthine.

Skin sensitization

Anatoxin, ambigol, and the microcystin group, lyngbyatoxin A, lyngbyatoxin B, nodularin-R, and saxitoxin were predicted to cause skin sensitization (Tab. 5). In case a susceptible individual is exposed to a contact allergen, upregulation and clonal expansion of allergen-responsive T-cells occurs (Gilmour *et al.*, 2019).

Bioactivity of cyanotoxins

The computed values of different parameters of the isolated compounds are summarized in Tab. 6. We found that Lyngbyatoxin shows activity through the protease inhibitor, the G-protein-coupled receptor (GPCR ligand), the GPCR ligand, and the kinase inhibitor. In clinical medicine, GPCR ligands became the most successful molecular drug targets. Antagonists, as well as agonists of the GPCR class, were applied in the treatment of every major organ system, including the respiratory, metabolic, urogenital, and cardiovascular systems. Considering their widespread expression and important regulatory and mechanistic characteristics, GPCRs have manifold functions in living beings (Insel *et al.*, 2007; Campbell and Smrcka, 2018). Our study predicted anatoxin-a and lyngbyatoxin to affect GI absorption and BBB penetration. Factors ensuring overall drug absorption include compound solubility and permeability as well as compound dissolution and gastrointestinal conditions, drug- and formulation- related parameters, drug product dissolution, as well as active transport, passive diffusion, and metabolism of toxicants of drugs (Freerks *et al.*, 2019).

TABLE 4

hERG-predictions of 20 cyanotoxins from Pred-hERG 4.2

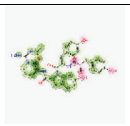
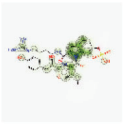
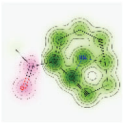
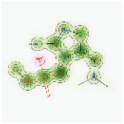
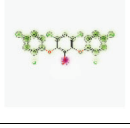
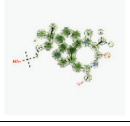
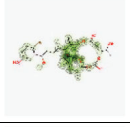
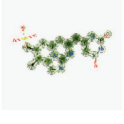
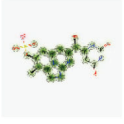
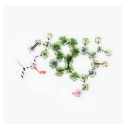
Compounds	Prediction	Potency	Applicability domain (AD)	
			Results	Probability
Aeruginosin 103-A	Potentially cardiotoxic (+) 50%	Weak or moderate 50%	Yes Value = 0.27 Limit = 0.26	
Aeruginosin 98-b	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.25 Limit = 0.26	
Anatoxin-a	Non-cardiotoxic (–) 60%	Not applicable	No Value = 0.23 Limit = 0.26	
Anatoxin-a(s)	Non-cardiotoxic (–) 70%	Not applicable	No Value = 0.22 Limit = 0.26	
Ambigol A	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	Yes Value = 0.29 Limit = 0.26	
Ambigol B	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	Yes Value = 0.3 Limit = 0.26	
Ambigol C	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	No Value = 0.22 Limit = 0.26	
Aplysiatoxin	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.22 Limit = 0.26	
Deoxycylindrospermopsin	Non-cardiotoxic (–) 50%	Not applicable	No Value = 0.22 Limit = 0.26	
7-Epi-cylindrospermopsin	Non-cardiotoxic (-) 50%	Not applicable	No Value = 0.22 Limit = 0.26	
Lyngbyatoxin A	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	No Value = 0.23 Limit = 0.26	
Lyngbyatoxin B	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	No Value = 0.23 Limit = 0.26	

Table 4 (continued).

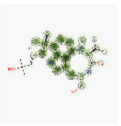
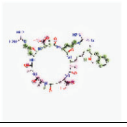
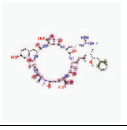
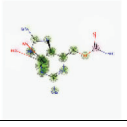
Compounds	Prediction	Potency	Applicability domain (AD)	
			Results	Probability
Lyngbyatoxin C	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	No Value = 0.22 Limit = 0.26	
Microcystin-LR	Potentially cardiotoxic (+) 60%	Weak or moderate 60%	No Value = 0.24 Limit = 0.26	
Microcystin-RR	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.24 Limit = 0.25	
Microcystin-YR	Potentially cardiotoxic (+) 50%	Weak or moderate 50%	No Value = 0.25 Limit = 0.26	
Microcystin-LA	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.25 Limit = 0.26	
Nodularin	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.26 Limit = 0.26	
Nodularin-R	Potentially cardiotoxic (+) 60%	Weak or moderate 50%	No Value = 0.24 Limit = 0.26	
Saxitoxin	Non-cardiotoxic (-) 70%	Not applicable	No Value = 0.22 Limit = 0.26	

TABLE 5

Skin sensitization predictions of cyanotoxins

Compounds	Human skin sensitization		Murine local lymph node assay (LLNA)		Direct peptide reactivity assay (DPRA)		Human cell line activation test (h-CLAT)		KeratinoSensTM		FINAL RESULT (consensus model)	
	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence
Aeruginosin 103-A	Sensitizer	60%	Non-Sensitizer	60%	Non-Sensitizer	50%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	70%
Aeruginosin 98-b	Non-Sensitizer	60%	Non-Sensitizer	60%	Non-Sensitizer	70%	Non-Sensitizer	70%	Non-Sensitizer	50%	Non-Sensitizer	80%
Anatoxin-a	Non-Sensitizer	50%	Non-Sensitizer	70%	Sensitizer	80%	Sensitizer	80%	Sensitizer	70%	Sensitizer	80%
Anatoxin-a(s)	Sensitizer	80%	Sensitizer	50%	Sensitizer	80%	Non-Sensitizer	50%	Sensitizer	80%	Sensitizer	60%
Ambigol A	Sensitizer	70%	Sensitizer	80%	Sensitizer	60%	Sensitizer	70%	Non-Sensitizer	60%	Sensitizer	90%
Ambigol B	Sensitizer	60%	Sensitizer	80%	Sensitizer	60%	Sensitizer	90%	Non-Sensitize	50%	Sensitizer	90%
Ambigol C	Sensitizer	60%	Sensitizer	90%	Sensitizer	60%	Sensitizer	90%	Non-Sensitize	50%	Sensitizer	90%

(Continued)

Table 5 (continued).

Compounds	Human skin sensitization		Murine local lymph node assay (LLNA)		Direct peptide reactivity assay (DPRA)		Human cell line activation test (h-CLAT)		KeratinoSensTM		FINAL RESULT (consensus model)	
	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence	Results	Confidence
Aplysiatoxin	Sensitizer	70%	Non-Sensitizer	60%	Sensitizer	50%	Non-Sensitize	70%	Sensitizer	90%	Non-Sensitize	80%
Deoxycylindrospermopsin	Non-Sensitizer	60%	Non-Sensitizer	80%	Sensitizer	60%	Sensitizer	60%	Non-Sensitize	70%	Sensitizer	60%
7-Epi-cylindrospermopsin	Non-Sensitizer	80%	Non-Sensitizer	80%	Non-Sensitizer	50%	Non-Sensitizer	70%	Non-Sensitizer	80%	Non-Sensitizer	80%
Lyngbyatoxin A	Sensitizer	90%	Non-Sensitizer	60%	Sensitizer	70%	Sensitizer	70%	Sensitizer	60%	Sensitizer	80%
Lyngbyatoxin B	Sensitizer	80%	Non-Sensitizer	60%	Sensitizer	80%	Non-Sensitizer	60%	Non-Sensitizer	50%	Non-Sensitizer	50%
Lyngbyatoxin C	Sensitizer	80%	Non-Sensitizer	60%	Sensitizer	80%	Sensitizer	60%	Sensitizer	60%	Sensitizer	70%
Microcystin-LR	Sensitizer	70%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	60%	Sensitizer	70%	Sensitizer	50%
Microcystin-RR	Sensitizer	70%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	60%	Sensitizer	70%	Sensitizer	50%
Microcystin-YR	Sensitizer	70%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	60%	Sensitizer	70%	Sensitizer	50%
Microcystin-LA	Sensitizer	70%	Non-Sensitizer	60%	Sensitizer	70%	Non-Sensitizer	60%	Sensitizer	70%	Sensitizer	60%
Nodularin	Sensitizer	60%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	60%	Sensitizer	70%	Non-Sensitizer	50%
Nodularin-R	Non-Sensitizer	50%	Non-Sensitizer	70%	Sensitizer	60%	Non-Sensitizer	50%	Sensitizer	70%	Sensitizer	50%
Saxitoxin	Sensitizer	60%	Non-Sensitizer	70%	Sensitizer	70%	Sensitizer	70%	Sensitizer	70%	Sensitizer	60%

TABLE 6

Bioactivity potential results as predicted by Molinspiration

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Aeruginosin 103-A	−0.03	−1.08	−0.89	−0.90	0.71	−0.56
Aeruginosin 98-b	0.49	−0.64	−0.51	−0.57	1.06	0.21
Anatoxin-a	−0.44	0.51	−0.99	−0.84	−0.49	−0.15
Anatoxin-a(s)	0.82	1.11	0.36	−0.21	0.60	1.25
Ambigol A	0.10	−0.12	0.16	0.28	0.00	0.07
Ambigol B	0.01	−0.08	0.10	0.09	−0.05	0.05
Ambigol C	−0.00	−0.17	0.16	0.05	−0.09	−0.02
Aplysiatoxin	0.05	−0.40	−0.02	0.11	0.09	0.28
Deoxycylindrospermopsin	0.82	−0.07	−0.39	−0.55	0.59	0.65
7-Epi-cylindrospermopsin	0.82	−0.13	−0.35	−0.45	0.54	0.65
Lyngbyatoxin A	0.49	0.20	0.42	0.06	0.36	0.35
Lyngbyatoxin B	0.49	0.19	0.42	0.23	0.36	0.42
Lyngbyatoxin C	0.61	0.17	0.51	0.15	0.36	0.41
Microcystin-LR	−3.55	−3.73	−3.76	−3.73	−3.16	−3.61
Microcystin-RR	−3.64	−3.78	−3.81	−3.79	−3.49	−3.68
Microcystin-YR	−3.65	−3.79	−3.82	−3.79	−3.52	−3.69
Microcystin-LA	−3.08	−3.62	−3.63	−3.56	−2.48	−3.29
Nodularin	−1.87	−3.05	−3.03	−2.86	−1.10	−2.30
Nodularin-R	−1.69	−2.98	−2.88	−2.77	−0.92	−2.09
Saxitoxin	0.38	0.22	−0.18	−0.17	0.54	0.27
Bioactivity:	Active		Moderately active		Inactive	

Conclusion

Chemcomputation adds to the instrumentation applied in ecotoxicology, such as the prediction of the physiological effects of cyanotoxins. *In silico* tools add objectivity combines several approaches in repeatable and intelligent ways. Economic demands and fast technological progress are in favor of chemcomputational tools. Cheminformatics allows for higher throughput and constant optimization. Cheminformatic approaches have a higher reproducibility, are less time consuming, and are less expensive. Computational approaches can also prioritize chemicals in order to reduce the amount of costly *in vivo* and *in vitro* toxicological screening and provide early alerts for unexplored, newly discovered, or newly developed substances. Through their replacement, they reduce the use of experimental efforts.

A lack of transparency and quality of the training set of experimental data provide some limitations. For example, carcinogenicity prediction can only be applied with non-genotoxic compounds. Neurotoxicity, hepatotoxicity, and developmental toxicity cannot be accurately predicted by *in silico* methods. These are complex phenomena with multiple endpoints, unlike issues with well-understood mechanisms like mutagenicity, sensitization, and aquatic toxicity – as exemplified in this study by cyanotoxins.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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